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STUDIES ON THE SEED OIL OF ABRUS PRECATORIUS LINN

Part II.—Composition of the Lipid Classes

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The seed oils of white, scarlet and the black varieties of *Abrus precatorius* Linn were studied for their lipid composition with the help of TLC and GLC. Each oil was first separated by TLC into the respective lipid classes which were transmethylated and analysed by GLC for their fatty acid composition. The weight percentages of TG in the white, scarlet and the black varieties were 39.2, 25.3 and 40.6% respectively. The lower percentage of TG in the scarlet variety is probably due to incomplete enzymic esterification of the DG, which is higher in this variety (13.5%). The black variety contained the highest amount of FFA (35.5%), showing the possibility of higher activity of lipolytic enzymes. In the individual lipid classes, palmitic and behenic acids were the major saturated fatty acids. Amongst the unsaturated acids, oleic and eicosenoic acids were most predominent, while linoleic was present in appreciable amounts. Linolenic acid was present in higher proportions in the DG, MG and SE fractions of scarlet and MG, SE and PL fractions of the black variety. Presence of odd numbered C15:0 and branched chain C18:0 acids in the oils could have originated from microorganisms, a number of which have already been reported in the seeds of *Abrus precatorius* Linn.

On the basis of the total fatty acid composition of *Abrus precatorius* Linn[1] and in accordance with the classification of Roxburg[2] it seems probable that there are three principal varieties of *Abrus precatorius Linn.*—white, scarlet and black—the other four varieties reported in the last communication are, in one way or the other, related to these three varieties. It was therefore thought of interest to study the various lipid classes of the white, scarlet and the black varieties in greater detail which is being discussed in the present investigation.

Materials and Methods

Oil was extracted from the powdered seeds with a mixture of chloroform—methanol (2:1 v/v) as described earlier.[1] The separation of the various lipid classes was achieved by TLC on plates (20×20 cm) coated with silica gel G (Merck), 0.25 mm thick. The plates were activated at 110°C for 1 hr, and washed with methanol to remove any lipids or other impurities prior to the application of the sample.

Each oil sample (25 to 35 mg) was applied on the plates in the form of a band together with standard reference compounds, and developed with a mixture of ether, pertoleum ether and acetic acid (20:80:1 v/v). After development, the plates were dried in air and the bands made visible with the help of iodine vapours (Fig. 1). The separated lipids were marked and scraped off the plates and quantitatively collected in screw-capped tubes for

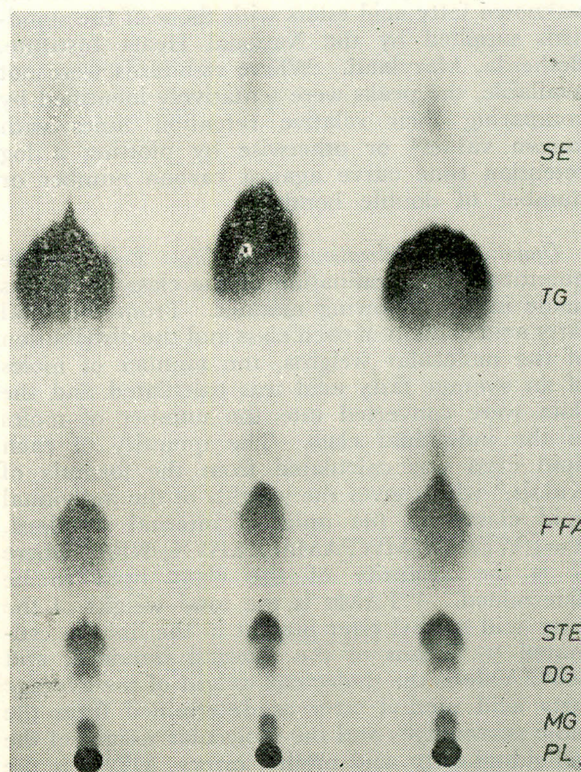

Fig. 1.—Separation of the Seed Oil of *Abrus precatorius* Linn into lipid classes by T.LC. SE—steryl esters; TG—triglycerides; FFA—free fatty acids; STE—sterols; DG—diglycerides; MG—monoglycerides; PL—phospholipids.

transmethylation. All the lipid fractions excepting the sterols, were transmethylated using the method of Ways *et al.*[3] with slight modifications.

To each tube containing the individual lipid fractions, a solution of 0.1 mg heptadecanoate in 1 ml methanol was added as an internal standard, followed by 1.5 ml 20% methanolic sulphuric acid. The tubes were then tightly secured with Teflon screw caps and heated in an oven at 80°C for 2 hr. After cooling, 1 ml distilled water was added to each tube and the methyl esters were extracted quantitatively with petroleum ether (b.p. 40–60°C). The extract was concentrated and transferred into small tubes with the help of Pasteur's pipette and the solvent removed by flushing with nitrogen. The methyl esters so obtained were kept in sample tubes and analysed, as soon as possible, for their fatty acids by GLC.

Gas Liquid Chromatography.—Gas liquid chromatography of the various lipid fractions was made on a Varian Aerograph model—600, gas chromatograph equipped with flame ionisation detector, as described before.[1] Identifications were based on comparison of retention times of the unknown fatty acid esters with those of the standards supplied by the National Heart Institute, Bethesda, Maryland. Where standards were not available, the peaks were tentatively identified by comparing their relative retention times with known values[4] or otherwise by plotting a log retention time curve against carbon number or number of double bonds.[5]

Quantitative Estimation of the Class Lipids.—The quantitative determination of the class lipids was made in the following manner. From the total fatty acid content of each class and the distribution of the molecular weights, the number of moles of an average fatty acid was calculated and the data were converted into the number of moles of the individual class. The quantity of each lipid class was calculated from the formula of Kuksis,[6] which gives the weights of the individual lipid classes in the units of internal standard. $X=\{(WFAMe/MWFAMe)\times MWX\}/NFA$, where X is the quantity of the class lipid to be determined. WFAMe is the total weight of the fatty acid methyl ester present in the lipid as estimated by means of the internal standard, and MWFAMe is the molecular weight of the average fatty acid methyl ester as determined from the mole percentages and the molecular weight of the individual fatty acid methyl esters; MWX is the molecular weight of the lipid molecule containing the average molecular weight fatty acid characteristic of each sample; and NFA is the number of average fatty acid residues per molecule of the lipid class. The calculations of phospholipids and the steryl esters were made as lecithin and β-sitosteryl esters respectively.

Discussion

As mentioned earlier,[1] samples for the present investigation were procured from three different stores, thus the possibility of having seeds of different ages in each variety may not be ignored. It is also possible that a particular lot may be composed of relatively large number of mature seeds, while other lots of the same variety may have different proportions of mature and immature seeds. Thus the results may not be representative of a particular age group, but of seeds under various stages of maturity. The present study, therefore, is a report on the oils of seeds present in a particular lot at the time of sampling.

Separation of the seed oil of *Abrus precatorius* Linn into various lipid classes is shown in Fig. 1. Seven spots representing the steryl esters (SE), triglycerides (TG), free fatty acids (FFA), sterols (STE), diglycerides (DG), monoglycerides (MG) and phospholipids (PL) were detected.

The amounts of various lipids present in the three principal varieties of *Abrus precatorius* Linn, expressed as weight per cent of the total are shown in Table 1. It is evident that the white and black varieties have higher proportions of triglycerides (39.2 and 40.6% respectively) than the scarlet variety (25.3%). On the other hand, higher percentage of diglycerides (13.5%) is present in the scarlet variety. It is possible that lesser amount of triglycerides in this variety is due to incomplete enzymic esterification of the diglycerides. The black variety has minimum quantities of monoglycerides and steryl esters (3.0 and 3.9% respectively), but the highest amount

TABLE 1.—LIPID COMPOSITION OF THE SEED OIL ISOLATED FROM *Abrus precatorius* LINN.

Lipid class	Types		
	White (wt %)	Scarlet (wt %)	Black (wt %)
Triglycerides	39.2	25.3	40.6
Diglycerides	7.3	13.5	4.9
Monoglycerides	7.4	7.3	3.0
Free fatty acids	21.5	17.3	35.5
Steryl esters (as β-sitosteryl esters)	8.8	15.4	3.9
Phospholipids (as lecithin)	15.8	21.0	12.1

TABLE 2.—FATTY ACID PATTERN OF LIPID CLASSES* IN THE OIL OF *Abrus precatorius* LINN.

Fatty acids **	White (wt %)						Scarlet (wt %)						Black (wt %)					
	TG	DG	MG	FFA	SE	PL	TG	DG	MG	FFA	SE	PL	TG	DG	MG	FFA	SE	PL
Saturated																		
$C_{14:0}$	tr	1.8	2.6	1.6	9.2	1.6	tr	1.2	1.0	tr	2.5	0.8	tr	tr	tr	tr	tr	tr
$C_{15:0}$[a]	x	1.8	2.5	1.8	1.9	1.7	x	1.4	0.5	x	0.3	x	x	x	x	x	x	x
$C_{16:0}$	10.9	9.0	10.2	8.3	16.0	12.4	11.0	8.3	5.6	15.7	10.1	12.8	10.6	11.3	10.9	13.9	10.4	9.9
$C_{18:0}$br.	tr	4.1	12.6	4.3	7.7	5.8	tr	0.3	4.8	tr	2.0	1.4	1.5	1.7	3.6	tr	2.8	1.7
$C_{18:0}$	3.6	0.7	5.0	3.2	8.2	3.1	4.1	1.4	2.8	6.4	4.0	3.3	5.2	3.0	2.5	3.8	1.5	3.1
$C_{22:0}$	7.8	10.2	1.2	6.4	tr	6.8	7.4	4.8	4.8	5.7	4.5	3.5	8.2	8.6	3.8	7.0	4.8	2.9
$C_{24:0}$	4.1	x	x	2.0	x	1.4	4.2	x	x	1.8	x	x	6.4	2.8	x	3.8	x	x
Total	26.4	27.6	34.1	27.6	43.0	32.8	26.7	17.4	19.5	29.6	23.4	21.8	31.9	27.4	20.8	28.5	19.5	17.6
Unsaturated																		
$C_{16:1}$	2.1	3.8	7.7	7.6	5.8	6.8	2.0	0.5	0.7	0.7	1.5	1.1	1.0	1.1	1.4	0.4	1.0	1.4
$C_{18:1}$	41.2	46.7	22.5	34.3	27.6	38.3	45.0	36.7	26.5	48.2	11.1	47.2	32.5	33.7	37.9	51.6	18.9	32.6
Unknown	x	x	8.4	2.0	x	x	x	1.3	1.5	x	x	1.2	1.7	1.3	2.4	1.5	1.9	2.0
$C_{18:2}$	8.0	1.5	1.2	5.3	4.0 } b 3.7 }	1.3	5.6	2.4	tr	6.3	1.1	2.0	2.5	2.6	1.7	1.5	tr	tr
$C_{18:3}$	0.6	4.8	3.0	4.2	5.5	4.6	tr	21.4	36.8	tr	46.0	12.4	0.5	8.1	20.0	0.7	42.4	20.2
$C_{20:0}$ } $C_{20:1}$) }	19.6	5.8	9.8	6.1	3.6	4.9	16.6	14.4	9.6	12.0	8.6	11.3	20.6	18.4	4.7	11.1	11.8	15.5
Unknown	x	x	x	x	x	x	1.5	3.8	1.0	x	1.8	0.7	0.8	1.5	5.9	1.2	3.2	5.6
$C_{20:2}$	1.1	5.6	6.9	2.8	x	6.4	x	0.2	2.9	0.4	3.6	0.5	1.9	1.6	1.1	1.1	tr	4.0
$C_{20:3}$	tr	x	tr	tr	4.8	x	x	x	x	x	1.6	0.5	1.0	x	3.0	x	x	1.1
$C_{22:1}$	1.0	0.5	5.0	4.6	tr	tr	1.4	0.9	1.5	0.6	1.3	x	1.0	0.8	x	0.7	0.6	x
$C_{22:2}$	x	3.7	tr	4.0	2.0	2.2	x	x	x	x	tr	1.3	2.1	1.7	1.1	0.6	0.7	x
$C_{22:3}$[a]	x	x	1.4	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
$C_{22:4}$[a]	x	x	x	1.5	x	2.7	1.2	x	x	2.2	x	x	2.5	0.8	x	1.1	x	x
Total	73.6	72.4	65.9	62.4	57.0	67.2	63.3	81.6	80.5	70.4	76.6	78.2	68.1	71.6	79.2	71.5	80.5	82.4

*The short hand designation of class lipids are represented as: TG=triglycerides; DG=digylcerides; MG=monoglycerides; FFA=free fatty acids; SE=steryl esters; PL=phospholipids; **The short hand designation adapted by Farquhar *et al.*[10]; *a* Tentative identification only; *b* Probably an isomer of $C_{18:2}$.

amount of free fatty acids (35.5%). Scarlet and white varieties, however, have lower percentages of free fatty acids (17.3 and 21.5% respectively) and relatively higher proportion of phospholipids (21.0 and 15.8% respectively). Whether such differences are due to specific composition of the three varieties or mainly because of enzyme inactivation is not yet clear. However, enzyme inactivated peas have been reported to contain less free fatty acids than the raw peas and had larger proportion of fatty acids bound as PL and neutral fat.[7]

The fatty acid composition of individual class lipids is given in Table 2. In each lipid class of the three varieties; palmitic, and behenic acids are the major saturated acids. In addition to these, appreciable amounts of stearic acid is also present in all these classes. Interesting to note is the branched chain $C_{18:0}$ acid, which is present in large amounts in the white variety and in relatively lesser amounts in scarlet and black. With the exception of TG, $C_{15:0}$ acid is also present in lesser amounts in the minor lipid classes DG, MG and SE of the scarlet variety, and absent in the black variety. Odd numbered and branched chain fatty acids are reported to be present in certain microorganisms.[8] The presence of branched chain $C_{18:0}$ acid and odd numbered $C_{15:0}$ acid in these varieties might have originated from the microorganisms, a number of which have already been reported in the seeds of *Abrus precatorius* Linn.[9]

Amongst the unsaturated acids, oleic and and eicosenoir acids form the major constituent of all the lipid classes in the oils of white, black and scarlet varieties, while palmitoleic and linoleic acids are present in appreciable amounts in the white, but in smaller amounts in the black and scarlet varieties with the exception of TG and FFA fractions of the scarlet in which the amount of linoleic acid is comparatively higher. Similarly, linolenic acid is present in higher proportions in the DG, MG and SE fractions of the scarlet and MG, SE and PL fractions of the black variety.

It will be seen from Table 2, that although the overall qualitative pattern of saturated and unsaturated fatty acids amongst the various lipid classes of the oils of *Abrus precatorius* Linn is very much the same, some quantitative differences are obvious regarding the levels of individual fatty acids. These differences are more prominent amongst the MG, DG and SE fractions and to a lesser extent in the major lipid classes i.e., TG, FFA and PL. Such variations in the proportions of individual fatty acids of Abrus seeds may be taken as true representactive of the three specific major varieties. However, these differences cannot be used as criteria for the identification of the three varieties, since the age of the seeds could not be ascertained prior to the analysis.

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